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Insula-Specific ^{1}H Magnetic Resonance Spectroscopy Reactions in Heavy Smokers under Acute Nicotine Withdrawal and after Oral Nicotine Substitution

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Key Words
Insular cortex · Magnetic resonance spectroscopy · Nicotine · Nicotine withdrawal · Smoking addiction

Abstract
The aim of this study was to clarify whether addiction-specific neurometabolic reaction patterns occur in the insular cortex during acute nicotine withdrawal in tobacco smokers in comparison to nonsmokers. Fourteen male smokers and 10 male nonsmokers were included. Neurometabolites of the right and the left insular cortices were quantified by magnetic resonance spectroscopy (MRS) on a 3-Tesla scanner. Three separate MRS measurements were performed in each subject: among the smokers, the first measurement was done during normal smoking behavior, the second measurement during acute withdrawal (after 24 h of smoking abstinence), and the third shortly after administration of an oral nicotine substitute. Simultaneously, craving, withdrawal symptoms, and CO levels in exhaled air were determined during the three phases. The participants in the control group underwent the same MR protocol. In the smokers, during withdrawal, the insular cortex showed a significant increase in glutamine (Gln; $p = 0.023$) as well as a slight increase not reaching significance for glutamine/glutamate (Glx; $p = 0.085$) and a nonsignificant drop in myoinositol (mI; $p = 0.381$). These values tended to normalize after oral nicotine substitution treatment, even though differences were not significant: Gln ($p = 0.225$), Glx ($p = 0.107$) and mI ($p = 0.810$). Overall, the nonsmokers (control group) did not show any metabolic changes over all three phases ($p > 0.05$). In smokers, acute nicotine withdrawal produces a neurometabolic reaction pattern that is partly reversed by the administration of an oral nicotine substitute. The results are consistent with the expression of an addiction-specific neurometabolic shift in the brain and confirm the fact that the insular cortex seems to play a possible role in nicotine dependence.

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Introduction

Nicotine abuse is the leading cause of avoidable death and the most important cause of morbidity worldwide [1–3]. Although many patients who are addicted to nicotine would like to cease smoking, less than 50% are actually able to achieve sustained abstinence from smoking [4, 5]. The reasons for nicotine dependence are manifold and are only partially understood. In addition to known social, environmental, and psychological prerequisites, a genetic predisposition appears to be associated with a greater risk of nicotine dependence [6–9]. Investigations conducted in persons addicted to nicotine showed that chronic nicotine abuse may cause significant morphological changes in various structures of the brain, including the insular cortex [10, 11]. In contrast to other imaging modalities such as positron emission tomography, functional magnetic resonance imaging (MRI), or conventional MRI of the brain, ^{1}H-MR spectroscopy (MRS) enables the investigator to measure biochemical changes in a specific region of the brain. Especially neurometabolic reactions in the insular cortex are of great interest because the insula contributes significantly to central processing of cognitive, social, and emotional stimuli [12]. A noteworthy, recently published study in *Science* proved that nicotine addiction disappeared spontaneously in patients suffering from trauma with associated morphological damage within the insular cortex [13].

Consequently, based on this previous publication, the aim of the present prospective study was to investigate the neurometabolic role of the insular cortex with the aid of ^{1}H-MRS in subjects suffering from tobacco dependence. We intended to determine MRS changes during acute withdrawal and after oral treatment with a nicotine substitute compared to a nondependent control group. We hypothesized that the results will give a more detailed insight of the neurometabolic reaction of the brain, and more specifically of the insular cortex, to acute nicotine addiction and withdrawal.

Materials and Methods

Data Collection

This prospective clinical study, which was approved by the local institutional review board, was conducted from January 2011 to January 2012. All participants were given detailed information about the experimental procedure, and thereafter they provided written informed consent. To exclude possible hormonal effects during the menstrual cycle in women, only men were recruited. Further, participants taking medications such as opioids, sedatives or other psychopharmacological drugs were excluded. Thus, male persons >18 years of age, with no general contraindication to MRI investigation, were included in the study.

Study Population

Smokers

One hundred and fifty-one smokers were approached during their hospital stay. Of these, 135 heavy smokers refused to participate in the study. Two participants were subsequently excluded from the study because of noncompliance. Overall, 14 male smokers aged 50.43 ± 12.57 years (mean ± SD) were finally recruited for the investigation.

Smokers reported a mean duration (±SD) of smoking of 33.64 ± 13.93 years; they smoked at least 28.57 ± 10.27 cigarettes/day. Smokers reported a mean of 1.21 ± 1.48 previous attempts to quit smoking. None of the smokers had an underlying psychiatric disease or any other addiction, which was confirmed by a comprehensive psychiatric investigation. None of the participants consumed medications like opioids, sedatives, or similar drugs that potentially influence neurometabolic reactions. All subjects were given the option to participate in a smoking cessation program free of charge subsequent to the experiment. Sociodemographic and clinical characteristics of the study population are presented in tables 1 and 2.

Control Group

Staff members of our hospital, with no previous psychiatric disease, no nicotine addiction (nonsmokers along their whole life) and absence of addictive diseases, which was confirmed by a preceding psychiatric investigation, were recruited as controls. The participants also had no general contraindication to MRI. Thus, 10 male nonsmokers with a mean age of 36.6 ± 9.24 years were included in the study.

Psychometric Measures

Three separate MR experiments were performed, as described in detail later on. During the baseline session (1st MRI), participants completed self-report measures to assess the history and level of their nicotine dependence. The absence of psychiatric axis-I comorbidity was verified with the Structured Clinical Interview, which is based on DSM-IV criteria (American Psychiatric Association, 2000) [14–16]. The Fagerström Questionnaire for Nicotine Dependence was used to assess the level of nicotine dependence [15, 16].

Nicotine craving (2nd and 3rd MRI) was determined using a self-report with the Questionnaire of Smoking Urges (QSU) at baseline, after withdrawal and after nicotine treatment (German version) [17–19]. Participants provided a self-report of nicotine withdrawal symptoms using the Minnesota Nicotine Withdrawal Scale (MNWS) at baseline, withdrawal phase and after nicotine treatment [19].

First Measurement (Baseline)

Smokers and nonsmokers were examined using MRI. Additionally, all participants in the smoker group were asked to complete a Fagerström Questionnaire for Nicotine Dependence, MNWS and QSU. An attending psychiatrist explained how the questionnaires had to be filled in.

Table 1. Demographic data of the smokers and nonsmoking controls

Parameters	Smokers (n = 14)	Nonsmokers (n = 10)
Age, years	50.43 ± 12.57	36.6 ± 9.24
Cigarettes smoked on baseline day, n	22.21 ± 8.64	0 ± 0
Exhaled CO at baseline, ppm	23.21 ± 10.14	2.75 ± 2.63
FTND	7.07 ± 1.64	0.0
Cigarettes/day, n	28.57 ± 10.27	0
Age at smoking onset, years	16.79 ± 2.89	–
Years smoked, n	33.64 ± 13.93	0

Initial measurements in the smokers performed during normal smoking behavior yielded high CO values in exhaled air. FTND = Fagerström Test for Nicotine Dependence. The healthy control group, with no exposure to smoking, had normal respiratory gas values at the first as well as the second measurement. Means ± SD.

Table 2. Smokers: psychological and clinical information

Parameters	Baseline	Withdrawal phase	Nicotine substitute phase
Exhaled CO, ppm	23.21 ± 10.14	3.57 ± 1.95	–
MNWS	0.29 ± 0.61	10.71 ± 4.50	1.14 ± 1.17
QSU	104.21 ± 21.79	155.64 ± 31.10	110.86 ± 33.43

Smokers presented normal psychological values (craving) at baseline (during the initial phase with normal smoking behavior). Their urge to smoke (QSU) and nicotine withdrawal symptoms (MNWS) increased significantly after 24 h of withdrawal ($p < 0.001$), and was obviously reduced directly after oral administration of a nicotine substitute (phase 3). CO values fell significantly after 24-hour withdrawal of tobacco ($p < 0.001$). Means ± SD.

Members of the smokers' group were asked to continue their usual smoking behavior on the 1st day of the experiment. To verify smoking behavior, participants underwent expired carbon monoxide measurements at baseline. The CO breath test was measured before MRS (see technical details below).

The control group (nonsmokers) pursued their usual daytime obligations and a CO breath test was also performed.

Second Measurement ('Stimulation Phase' after Withdrawal)

Smokers and nonsmokers were reexamined with MR within 5 days according to possible free slots in our scanner management. Smokers had to completely abstain from smoking for 24 h prior to the second measurement. Before starting the MR investigation, the MNWS and QSU were again filled in by all study participants in order to document their current state of well-being and level of craving. CO values in expired air were measured again in order to document the withdrawal status and confirm compliance. Participants with CO values >5 mg/dl were excluded from the study as we assumed that they were noncompliant and had smoked in between, thus violating the previous agreement.

Nonsmokers did not change any habits and underwent the identical psychometric tests as well as the second MR examination.

Third Measurement (after Stimulation: Substitution/ Treatment with Nicotine Tablets)

After completion of the second MR measurement, we removed the study participants from the MR device for 40 min. Immediately after MRI, members of the smoking group received a sublingual nicotine tablet (Nicorette®, 2 mg; Janssen-Cilag AG, Baar, Switzerland), which they placed under the tongue in order to dissolve for 20 min. The nonsmokers could relax and were permitted to drink a glass of water between the two MR examinations. No nicotine tablet was given to the nonsmokers.

Members of both groups were then asked to fill in the questionnaires once again in order to record the changes in their craving and basic psychological status.

Thereafter, smokers and controls were investigated a third time by MRS. The entire experiment is summarized in a schematic diagram (fig. 1).

Experimental Investigations by ^{1}H MRS

The subjects were positioned supine in a 3-Tesla MR unit (Achieva, release 2.6.1; Philips Healthcare, Best, The Netherlands). A common 4-channel T/R head coil (Philips Healthcare) was used. To prevent involuntary head movements, small foam cushions were placed between the head coil and the volunteer's

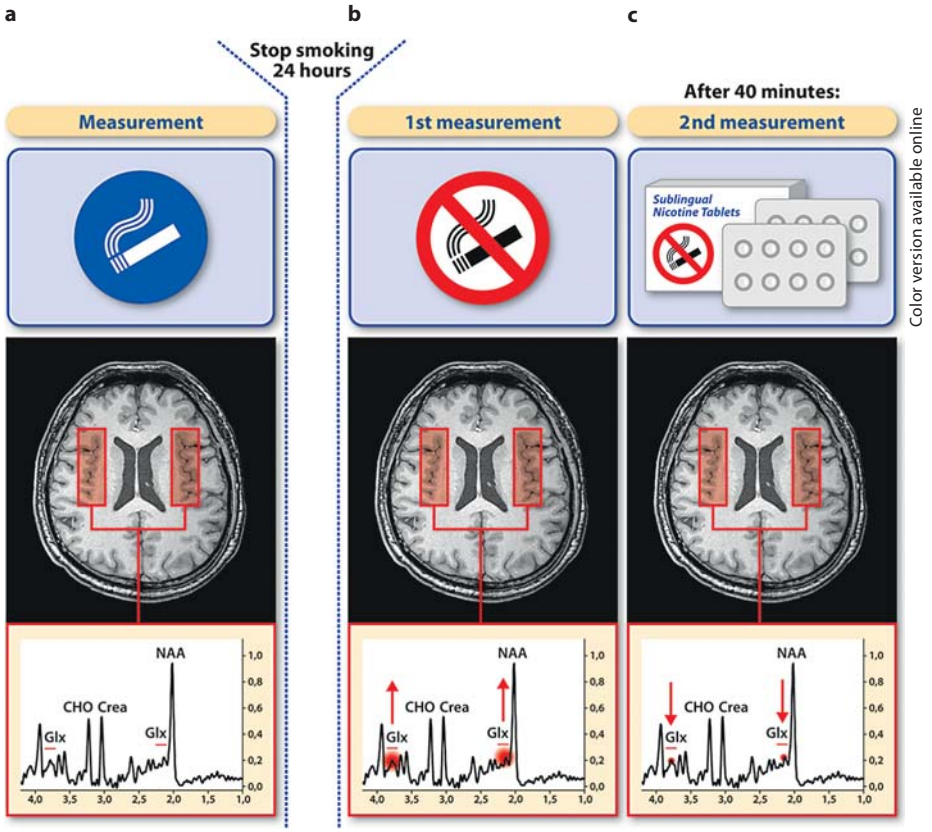

Fig. 1. Experimental setup for the smoker group. In phase 1, smokers demonstrate 'normal' smoking behavior (**a**). After 24 h of tobacco withdrawal, the insular cortex was reinvestigated by MRS during pronounced acute withdrawal symptoms (phase 2; **b**). To relieve withdrawal symptoms, smokers were given an oral nicotine substitute 40 min before the subsequent third MRS measurement (phase 3; **c**). CHO = Choline; Crea = creatine; NAA = N-acetyl aspartate.

head. The standardized MR protocol consisted of a scout, a reference scan, and a transverse, sagittal and coronary T1-weighted morphological sequence, followed by ^{1}H spectroscopic measurements (table 3).

The left and the right insular regions were measured using the ^{1}H spectroscopy PRESS sequence (point-resolved spectroscopy). Volumes of interest (determined for each subject) were placed over the entire insula by an experienced board-certified radiologist with 13 years of neuroradiological experience including MRS investigations of the insular cortex (investigator A. Gutzeit/blinded for review).

Table 3. MR pulse sequence parameters

Parameters	T1	^{1}H-MRS PRESS
TR, ms	600	2,000
TE, ms	10	30
Slice thickness, mm	4	–
Voxel size, mm	0.9 × 1.12	VOI: 20 × 20 × 37.5
NSA	1	96
Acquisition time, min	2.07	3.48

Postprocessing and Evaluation of MRS Data

For quantification, spectroscopic data were exported and analyzed with an LCModel using a basic spectrum for echo time (30 ms) and field strength (3 T) for relevant metabolites. The neurometabolites included choline, creatine, N-acetyl aspartate, glutamate (Glu), glutamine (Gln), Gln/Glu (Glx), myoinositol (mI), alanine, lactate, aspartate, and others. The technique we used has been described previously [20]. Fit was achieved within a chemical shift range between 0.5 and 4.2 ppm. All postprocessing results are calculated with the measured signal of unsuppressed water, which leads to absolute concentration (in mmol/l).

Statistical Analysis

Descriptive statistics are given as means ± SD. Data were analyzed using SPSS (version 20; IBM Corp., Armonk, N.Y., USA). The study design could not be equalized for smokers and nonsmokers because nonsmokers were naturally not able to undergo a pharmaceutical intervention which was comparable to that of the smokers. Thus, those two groups were analyzed separately with a repeated-measure general linear model (GLM) to analyze changes between the three measurements. The Mauchly test of sphericity was used to control for variance homogeneity and the Greenhouse-Geisser correction was applied where appropriate. Pairwise comparisons (phases 1 vs. 2; and 2 vs. 3) were also performed. Hence, the significance level was set at $\alpha = 0.025$ to correct for multiple comparisons (Bonferroni correction). To further reduce the number of comparisons, repeated-measure GLM and pairwise comparisons were run with the mean metabolite values between left and right sides. Overall, there were no significant differences between the right and left brain hemisphere. All other intragroup comparisons (CO) were analyzed with a paired-sample t test.

Table 4. Absolute MRS results for Gln, Glx, Glu, and mI: estimated marginal means and 95% confidence intervals (CI) for smokers and nonsmokers at time points 1–3

a Glutamine

Phase	Mean	95% CI
Smokers		
1	3.582	3.189–3.974
2	4.086	3.555–4.617
3	3.727	3.196–4.258
Nonsmokers		
1	2.519	1.740–3.298
2	2.658	2.090–3.226
3	2.780	2.150–3.410

There was no significant within-subject effect for the smokers ($F_2 = 1.867$, $p = 0.175$). Gln demonstrated a significant increase between baseline and withdrawal phase ($p = 0.023$). After administration of the nicotine substitute, Gln levels reversed, showing a minimal, nonsignificant drop ($p = 0.225$).

b Glutamine/glutamate

Phase	Mean	95% CI
Smokers		
1	10.424	9.735–11.114
2	11.106	10.333–11.879
3	10.463	9.663–11.264
Nonsmokers		
1	9.070	8.069–10.070
2	9.089	8.058–10.120
3	9.253	8.346–10.160

There was no significant within-subject effect for the smokers ($F_2 = 1.676$, $p = 0.207$). A minimal increase between phases 1 and 2 ($p = 0.085$) was noted, as well as a minimal drop between phases 2 and 3 ($p = 0.107$).

c Glutamate

Phase	Mean	95% CI
Smokers		
1	6.843	6.481–7.204
2	7.020	6.698–7.343
3	6.736	6.382–7.090
Nonsmokers		
1	6.551	6.061–7.041
2	6.430	5.866–6.995
3	6.473	6.082–6.864

For the smokers, the within-subject effect gave the following results: $F_2 = 0.289$, $p = 0.302$; pairwise comparisons between phases 1 and 2 ($p = 0.375$), and phases 2 and 3 ($p = 0.040$).

d Myoinositol

Phase	Mean	95% CI
Smokers		
1	5.508	5.033–5.983
2	5.330	5.014–5.645
3	5.371	4.994–5.749
Nonsmokers		
1	5.242	4.804–5.681
2	5.291	4.814–5.767
3	5.248	4.705–5.792

For the smokers, the within-subject effect gave the following results: $F_2 = 0.530$, $p = 0.595$; pairwise comparisons between phases 1 and 2 ($p = 0.381$), and phases 2 and 3 ($p = 0.810$).

Results

All single-voxel MRS measurements fulfilled the quality requirements and thus could be evaluated both in the smokers as well as the nonsmokers using the LCModel evaluation method.

The repeated-measure GLM found a significant within-subject effect for both QSU ($F_2 = 21.670$, $p < 0.001$) and MNWS ($F_2 = 71.686$, $p < 0.001$; Greenhouse-Geisser correction: $F_{1.129} = 71.686$, $p < 0.001$). Exhaled air CO levels decreased significantly between baseline and withdrawal phase (smoker group) 24 h later (baseline 23.21 ± 10.14; withdrawal phase 3.57 ± 1.95; $p < 0.001$). Concomitantly, the urge to smoke (QSU: baseline 104.21 ± 21.79, withdrawal phase 155.64 ± 31.10; $p < 0.001$), and nicotine withdrawal symptoms (MNWS: baseline 0.29 ± 0.61, withdrawal phase 10.71 ± 4.50; $p < 0.001$) increased significantly in the smoker group versus baseline in the withdrawal phase. After oral treatment with 2 mg of nicotine, the withdrawal symptoms and craving were significantly improved ($p < 0.001$) and returned almost to baseline levels (table 2). Members of the nonsmoker group had no relevant test changes or signs of addiction during the three measurements.

Metabolic Changes

The metabolite patterns of Glx, Gln, Glu and mI demonstrated a stimulation effect in both insular regions and are shown in table 4a–d. Insular levels of these metabolites changed during nicotine withdrawal compared with

healthy controls and as well when comparing within subjects. These metabolites partly returned to baseline after oral application of a nicotine substitute. All other documented brain metabolites, such as choline, N-acetyl aspartate, Glu, alanine, lactate and aspartate, showed no tendency and no significant changes and are not discussed further.

Metabolic Changes in the Smokers

Glutamine

Although the within-subject effect is not significant ($F_2 = 1.867$, $p = 0.175$), Gln demonstrated a significant increase between phases 1 (baseline) and 2 (withdrawal phase; $p = 0.023$) at group level. After administration of the nicotine substitute, levels of the metabolite reversed, showing a minimal, nonsignificant drop between phases 2 (withdrawal phase) and 3 (withdrawal with nicotine tablet; $p = 0.225$; table 4a).

Glutamine/Glutamate

Both the within-subject effect ($F_2 = 1.676$, $p = 0.207$) and the pairwise comparisons at group level were not significant. We observed a discrete increase between phases 1 and 2 ($p = 0.085$), and a nonsignificant drop between phases 2 and 3 ($p = 0.107$; table 4b).

Glutamate

The within-subject effect was not significant ($F_2 = 0.289$, $p = 0.302$), and pairwise comparisons at group level between phases 1 and 2 did not significantly differ ($p = 0.375$). A nonsignificant drop in Glu occurred between phases 2 and 3 ($p = 0.040$; table 4c).

Myoinositol

The within-subject effect was not significant ($F_2 = 0.530$, $p = 0.595$); there was only a slight drop during the withdrawal phase ($p = 0.381$) and a return to the baseline level after application of nicotine without achieving statistical significance ($p = 0.810$; table 4d).

Metabolic Changes in the Control Group

As expected, no significant changes in the above-mentioned three metabolites were observed in the control group between the three time points corresponding to the single MR experiments (table 4).

The attempt to correlate MRS data with any of the psychological tests was not feasible from the methodological point of view. The internationally standardized questionnaires have a completely different focus excluding direct comparison with MRS data.

Discussion

As nicotine dependence is widespread and has significant health-related sequelae for smokers, it constitutes a major problem for society as a whole. Although this type of addiction is widespread, it was recognized as an addictive disease only in the late 1980s [5, 21]. The reasons for tobacco dependence are manifold. In addition, social, psychological, environmental, and genetic factors play an important role [6, 7]. Achieving smoking cessation on a permanent basis can be extremely difficult [22, 23]. Better understanding of the pathophysiology of addiction and associated metabolic processes within the brain is thus of outmost importance.

Due to its numerous functions, the insular cortex appears to play a special role in the development of addiction [10, 11, 13]. Indeed, following traumatic injury of the insula, smokers were spontaneously able to cease smoking without any craving [13].

In accordance with these earlier findings, MRS is a promising diagnostic tool to analyze the neurometabolic activity of the insular cortex. Using MRS, the brain's endogenous substances and metabolites can be measured in a noninvasive manner, and conclusions can be drawn about physiological intracellular changes [24].

Recent studies have shown that specific stimuli such as pain or anxiety may markedly influence spectroscopic conditions in the brain metabolically [25, 26]. Furthermore, MRS is successfully used in various addictive disorders to elucidate their pathophysiology and withdrawal [27–29].

The present investigation showed that nicotine dependence causes shifts in levels of specific metabolites in the insular region during acute nicotine withdrawal (fig. 2). The changes specifically concern three metabolites: Gln, mI, and Glx. Changes in mI (drop between baseline and withdrawal phase) and Glx (increase between baseline and withdrawal phase) only show a tendency to a statistical difference. However, a significant increase in Gln was noted during acute nicotine withdrawal. This specific difference cannot be explained by previously published or our own results. The encountered changes were reversible after oral administration of a nicotine substitute. In contrast to the smoker group, the (nonsmoking) control group revealed an absolute stable metabolic level over all three phases demonstrating good reproducibility of our measurement technique and a well-designed study protocol.

Nevertheless, our data should be interpreted with great caution. They show that smoking withdrawal causes metabolic changes in the insula, which are partly re-

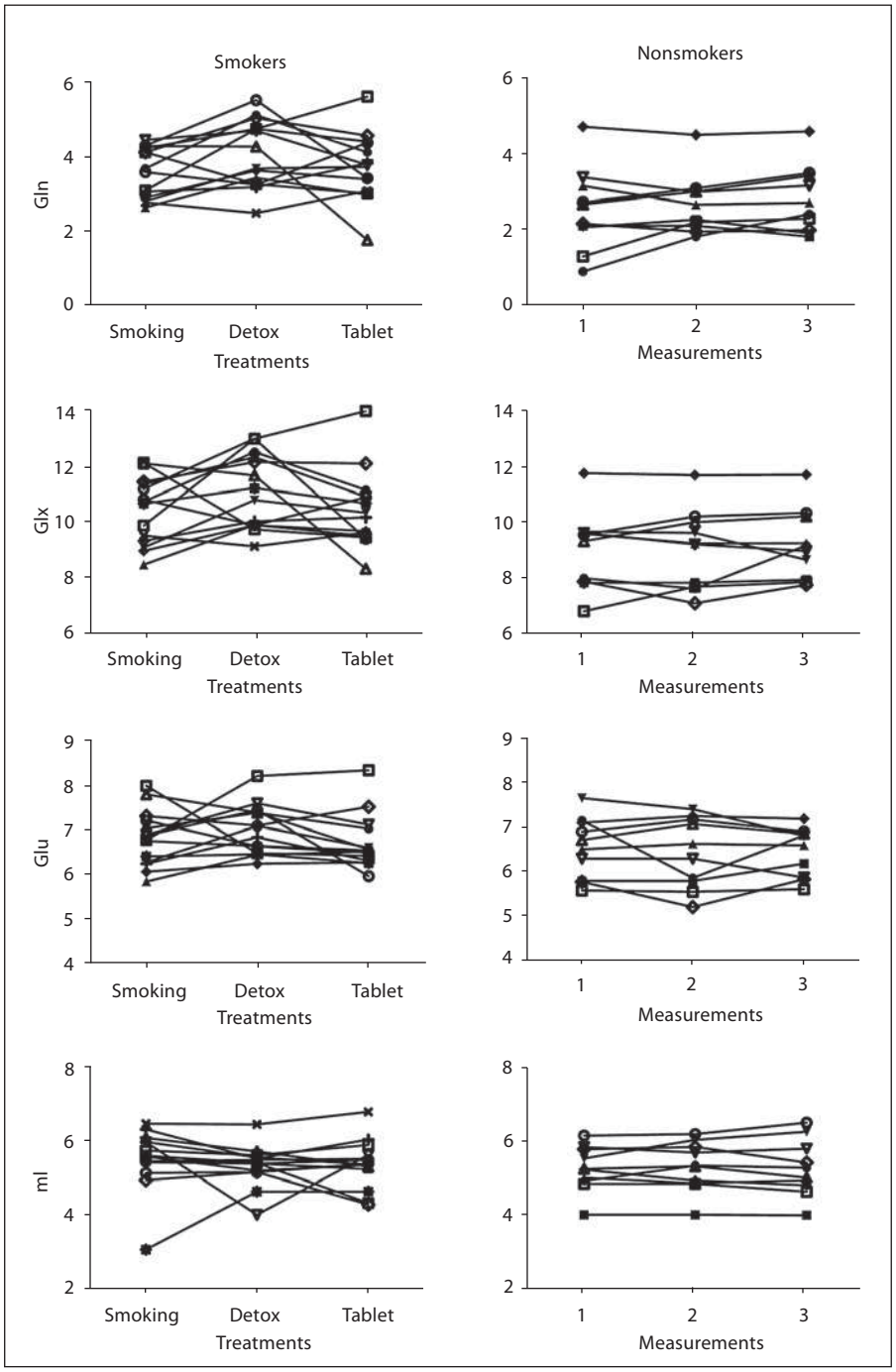

Fig. 2. Overview of MRS data: metabolites Gln, Glx, Glu, and mI for smokers (n = 14) during smoking, withdrawal (Detox), and after sublingual administration of a 2-mg nicotine tablet (left column) and for the control group (non-smokers; n = 10) during the first, second and third measurements (right column) are displayed. Due to the lack of a significant difference between the right and left hemispheres, neurometabolites are reported together.

versed by oral administration of a nicotine substitute. Indeed, due to the relatively low number of subjects included and the relatively low or nonexistent significance, it is still too early to define this pattern as an addiction-specific reaction of the brain. It is remarkable that the most abnormal neurometabolic values (compared to controls) are those obtained during withdrawal. One possible explanation is that the smoking state is the 'normal' state for those who are dependent. This could be a possible reason for the metabolic changes during withdrawal.

Moreover, the results appear comparable to other MRS investigations under different stress conditions. Hence,

these recently published studies showed that acute artificially produced dental pain causes metabolic shifts in the insula that were, in part, very similar to those observed in the present study [30, 31]. The aforementioned investigation showed increases in Gln and Glx as well as a drop in ml during the acute phase of pain, all of which resolved immediately after cessation of the external stimulus. Although the study designs with pain and here nicotine addiction differ strongly thus questioning comparison, the results do reveal marked similarities regarding metabolic dynamics in the 'stimulation phase' (nicotine withdrawal vs. pain stimulus) and the subsequent 'recovery phase' (decreasing withdrawal effects by the administration of oral nicotine vs. stopping the pain stimulus). It seems, even though not conclusive, that the brain reacts quite similarly in such stress situations at a neurometabolic level, especially for Gln and Glx. According to the physiological theory, Glu released from neurons is predominantly removed by astrocytic cells, which react by producing a constant Gln flow from astrocytes to neurons [32]. The question arises why much greater metabolic changes are observed in the insular region during acute pain compared to nicotine withdrawal, as recorded in our study: we presume that acute pain triggers a much stronger stress signal than nicotine withdrawal from a physiological point of view. This would explain the much more intense neurometabolic pattern within the brain. Moreover, regarding comparison, artificially induced pain can be much better standardized than a highly variable and rather subjective stimulus resulting from nicotine withdrawal. Pain impulses can be induced and stopped from one moment to the next, whereas nicotine withdrawal symptoms usually start slowly, and their severity and development are variable in individuals and over longer time periods. Furthermore, nicotine withdrawal does not merely entail a somatic component, but also involves psychological factors, which can be analyzed on the basis of questionnaires in an experiment of this nature, but cannot really be eliminated. All these different influencing factors must be taken into account when analyzing more profoundly neurometabolic behavior in patients suffering from addictive diseases.

Indeed, we cannot postulate a tobacco-specific reaction pattern due to the preliminary nature of our study. Nevertheless, we assume that nicotine withdrawal results in general neurometabolic stimulation. Overall this is speculative and cannot be proven. However, further studies will be needed to determine how metabolites in the insula react to other stressors such as hunger, cold, or other stressful stimuli, e.g. addiction.

We cannot explain the reason for the drop in mI and the rise in Gln and Glx observed in this study. Nevertheless, previous studies addressing addictive diseases and conditions such as alcohol withdrawal demonstrated comparable changes in specific metabolite levels with MRS [33]. Thus, neurometabolic changes might not only occur in the presence of nicotine withdrawal but possibly also in other types of withdrawal symptoms.

The metabolites Gln and Glu are important neurometabolic excitatory amino acids in the brain and thus one of the most important transmitter substances of the brain for any type of signal transmission. Gln results from the metabolic transformation of Glu, which is produced in astroglial cells of brain synapses. It is assumed that a large part of the brain's energy consumption is caused by the production of these metabolites alone [34–37]. This confirms once again our assumption: increases in these metabolite levels are possibly not a smoker-specific change, but a general sign of a 'stress-related neurotransmitter reaction', which is also observed in withdrawal. It must be emphasized that MRS quantification especially of Gln is still limited due to high standard deviations and technical limitations. Therefore, further interpretation of Gln must be done with appropriate caution. Nevertheless, the observed Gln shifts were only marked in the smokers whereas the nonsmokers did not show any difference among the three measurements.

For the time being, the minimal reduction in mI is difficult to explain from a biological point of view. To the best of our knowledge, no comparable descriptions in relation to addiction have been published. However, a similar drop in mI has also been noted in the acute phase of pain stimulation [25, 30, 31].

In addition to shifts in metabolite levels among smokers, as shown in the present study, the impact of tobacco on the structure of the brain is a well-known phenomenon. Several studies have shown that nicotine exerts a direct toxic effect on the brain reducing its volume [10, 38, 39]. How far the metabolic shifts observed in the present study are a consequence of this toxic impact cannot be stated with certainty. It would also be very interesting to determine how the spectra change in smokers while they undergo various withdrawal treatments consisting of psychotherapy, e.g. behavioral therapy. From a functional neurological perspective, the insular cortex is a very complicated structure, which is divided into anterior and posterior insular subdivisions. The posterior part is generally associated with sensorimotor function, including perception of pain, cold, heat, and other stimuli [12, 40–42]. In contrast, the anterior insula is not tra-

ditionally associated with somatosensory-related processing and seems to be rather involved in empathy and emotions [12, 43–45].

According to these functional subdivisions, the insular cortex in general might play an important role in nicotine addiction. Further studies investigating the effect of nicotine on this complicated part of the brain are required. Thus, it would be necessary to evaluate the metabolic reaction differentiating the discrete reactions of the anterior and posterior parts of the insular cortex under acute addiction.

There are several limitations of the present study. The group of smokers was rather small, possibly explaining the low significance recorded in the present study. Nevertheless, it should be emphasized that due to the pilot study methodology we did not have any databases for power analysis prior to the beginning of the investigation. One further limitation relates to the fact that we only measured metabolic reactions in the insular cortex. We thus cannot exclude the possibility that the metabolic changes might also occur in other brain areas. Moreover, MRS measurements are relatively time consuming; thus it is not possible to measure more than one brain region within an acceptable time. Furthermore, the control group consisting of healthy nonsmokers could not be given an oral nicotine substitute during the third phase because the local ethics committee objected to such an intervention. Therefore, the study conditions were not entirely identical for both groups, at least not during phase 3. Another possible limitation might be that we cannot exclude various external influences such as daytime or nutritional effects, for example. In addition, only male smokers and nonsmokers were included to prevent any hormonal influence, which might be another limiting factor. Finally, the control group and the smoker group were not matched for age. Thus, possible age-related structural brain differences could not be excluded as a possible source of metabolic differences.

Conclusions

Nicotine withdrawal is associated with significant increases in Gln levels in the insular cortex, which resolve after oral administration of a nicotine substitute. In the case of Glx and mI, changes are relatively discrete and nonsignificant under acute withdrawal, tending to reach baseline levels after oral administration of a nicotine substitute. Thus, it may be assumed that nicotine dependence is associated with specific reaction patterns, suggesting a pivotal role of the insular cortex.

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